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Bacteriological Quality of Mozzarella Cheese Sold in Tripoli Governorate

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Thirty samples of Mozzarella cheese (15 made traditionally from raw milk in Tripoli city (Libya) and other 15 imported samples sold in markets related to different brand names) were examined bacteriologically for their total bacterial count, psychrophilic count, coliform count (MPN/g), presumptive *Staphylococcus aureus* count, as well as enterococci count. Higher counts were found in locally manufactured Mozzarella cheese. Salmonellae were absent in all examined samples for both types, while *Escherichia coli* were isolated from 3 samples (20%) of locally made samples. According to the suggested Libyan Standards of such samples, most of examined locally manufactured Mozzarella cheese samples were found unacceptable.

Although Mozzarella cheese is one of several pasta filata cheeses that originated in Italy, it is consumed worldwide, largely due to the growth in popularity of pizza and similar foods (Bertola *et al*., 1996). Pasta filata cheeses are distinguished by a unique plasticizing and kneading treatment of the fresh curd in hot water, which imparts to the finished cheese its characteristic fibrous structure, melting and stretching properties (Fox, 1993).

It is very difficult to produce a high quality product using traditional method for manufacture of cheese due to lack of standardization of most manufacturing steps, especially the ripening conditions (Dervisoglu and Yazici, 2001). On the other hand, using pasteurized milk and starter culture in large scale production (imported type Mozzarella cheese) improves the keeping quality and significantly reduces pathogens in comparison with using raw milk in traditional method (Petracca *et al*., 1983). In General, Mozzarella cheese has a brief shelf-life (20 - 30 days) that depending on the extent and degree of refrigerated storage (Massa *et al.,* 1992; Kosikowski and Mistry, 1997).

Mostly, Mozzarella cheese is stored in refrigerator at 5°C in retail shops, fresh cheese is typically firm and has poor melting properties and although it will stretch, the overall texture will be unacceptable. However, as the cheese ages over a period of three weeks the texture becomes most suitable for preferable rheological properties (Rowney *et al.,* 1999). Mozzarella cheese stored for longer period becomes extremely soft and fluid when melted and is no longer acceptable for pizza (Kindstedt, 1993), in addition with such longer storage period, some bacterial population increases and much of which are undesirable, causing spoilage of the cheese or may be pathogenic, representing public health hazard to consumers.

Many outbreaks due to the consumption of Mozzarella cheese were reported (Salvatore *et al.,* 1992).

According to the suggested Libyan standards, the shelf-life of Mozzarella cheese stored in refrigerator (5°C) is one month in comparison with one year shelf-life for that stored at -18°C. Therefore, this work was planned to examine and compare the Mozzarella cheese made locally by traditional method in Tripoli city and that imported from different countries to the Libyan markets and its satisfaction to the known microbial limits suggested by Libyan Organization for Standardization.

Because only limited studies were previously conducted on Mozzarella cheese in Libyan Arab Jamahiriya, the aim of this study was to evaluate the bacterial quality of Mozzarella cheese sold in Libyan markets, either

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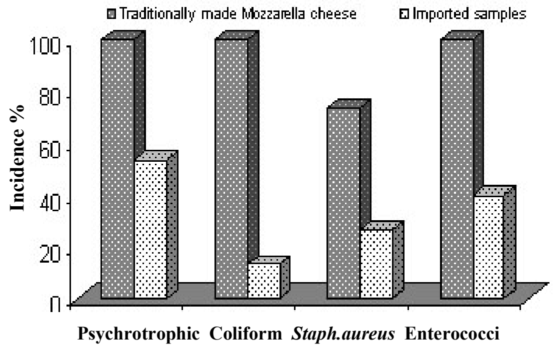


Fig. (1): Incidence percent of different examined microbial groups among examined samples of Mozzarella cheese.

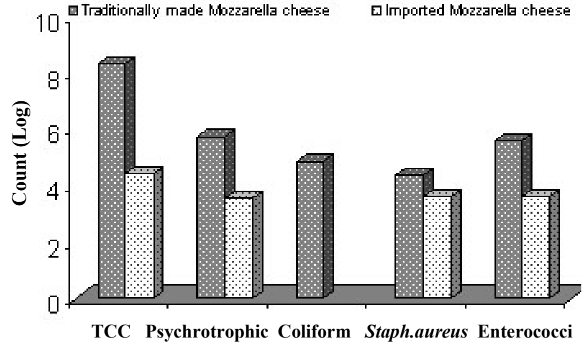


Fig. (2): Mean microbial group counts per gram "log" for positive samples of Mozzarella cheese made by both traditional method and imported samples.

Table (1): Statistical analytical results of different bacterial counts (CFU/g) in examined Mozzarella cheese samples made by traditional method. (No. of samples = 15).

Counts	Positive samples		Minimum	Maximum	Mean Count
	No.	%			
Total Colony Count	15	100	1.06×10^5	3.2×10^9	$8.5\times10^7 \pm 1.3\times10^7$
Psychrotrophic Count	15	100	3×10^4	4.8×10^7	$5.8\times10^5 \pm 0.65\times10^5$
Coliform Count	15	100	3.3×10^3	1.1×10^5	$7.3\times10^4 \pm 0.95\times10^4$
Staphylococcus aureus **(Presumptive count)**	11	73.33	30	1.7×10^6	$2.3\times10^4 \pm 0.27\times10^4$
Enterococci count	15	100	1.8×10^3	7.6×10^7	$3.8\times10^5 \pm 0.85\times10^5$

Table (2): Statistical analytical results of different bacterial counts (CFU/g) in examined Mozzarella cheese samples made by modern dairy plants (imported samples) (No. of samples = 15).

Counts	Positive samples		Minimum	Maximum	Mean Count
	No.	%			
Total Colony Count	15	100	2×10^5	6.5×10^7	$2.7\times10^6 \pm 0.44\times10^6$
Psychrotrophic Count	8	53.33	60	7.4×10^4	$3.7\times10^3 \pm 0.29\times10^3$
Coliform Count	2	13.33	2	1.6×10^3	NA*
Staphylococcus aureus **(Presumptive count)**	4	26.66	30	1.8×10^4	$4.2\times10^3 \pm 0.36\times10^3$
Enterococci count	5	33.33	40	3.1×10^4	$4.1\times10^3 \pm 0.31\times10^3$

*NA:No statistical analysis due to few positive samples.

locally made "traditional types", or of imported origin, thus providing a national data about bacteriological quality of such cheese.

Materials and Methods

Collection of samples. Thirty samples of Mozzarella cheese; 15 samples were obtained from dairy plant produce Mozzarella cheese from raw milk using traditional method and 15 samples of packed imported retail samples obtained from Tripoli markets. Samples were collected and transferred to the laboratory for bacteriological examination.

Preparation of samples and decimal dilutions. Sample preparation was performed according to the method described by (APHA, 1992).

Psychrotrophic count. It was determined in a similar method to that of APC except that plates were incubated at 7°C for 10 days (Cousin *et al.,* 1992).

Coliforms count (MPN/g). It was done using lauryl sulphate tryptone broth according to (APHA, 1992). Coliform isolates were biochemically identified according to (Krieg and Holt, 1984).

Enterococci count. It was made using ESD

Table (3): Frequency distribution of the different microbial counts in examined Mozzarella cheese made locally by traditional method.

Intervals	Bacterial counts (CFU/g)									
	Total colony count		Psychrotrophic count		Coliform count		*Staphylococcus aureus* count		Enterococci count	
	No.	%	No.	%	No.	%	No.	%	No.	%
<10	0	0	0	0	0	0	4	26.67	0	0
$10 - <10^{2}$	0	0	0	0	0	0	1	6.67	0	0
$10^{2} - <10^{3}$	0	0	0	0	0	0	2	13.33	0	0
$10^{3} - <10^{4}$	0	0	0	0	3	20	2	13.33	3	20
$10^{4} - <10^{5}$	0	0	1	6.67	8	53.34	3	20	2	13.33
$10^{5} - <10^{6}$	1	6.67	11	73.33	4	26.66	1	6.67	7	46.67
$10^{6} - <10^{7}$	4	26.66	2	13.33	0	0	2	13.33	2	13.33
$10^{7} - <10^{8}$	6	40	1	6.67	0	0	0	0	1	6.67
$10^{8} - <10^{9}$	4	26.67	0	0	0	0	0	0	0	0
Total	15	100	15	100	15	100	15	100	15	100

Table (4): Frequency distribution of the different microbial counts in examined imported Mozzarella cheese.

Intervals	Bacterial counts (CFU/g)									
	Total colony count		Psychrotrophic count		Coliform count		*Staphylococcus aureus* count		Enterococci count	
	No.	%	No.	%	No.	%	No.	%	No.	%
< 10	0	0	7	46.67	14	93.33	11	73.33	10	66.66
$10 - <10^{2}$	0	0	1	6.67	0	0	1	6.67	1	6.67
$10^{2} - <10^{3}$	0	0	2	13.33	0	0	0	0	1	6.67
$10^{3} - <10^{4}$	0	0	4	26.66	1	6.67	2	13.33	2	13.33
$10^{4} - <10^{5}$	0	0	1	6.67	0	0	1	6.67	1	6.67
$10^{5} - <10^{6}$	3	20	0	0	0	0	0	0	0	0
$10^{6} - <10^{7}$	10	66.67	0	0	0	0	0	0	0	0
$10^{7} - <10^{8}$	2	13.33	0	0	0	0	0	0	0	0
Total	15	100	15	100	15	100	15	100	15	100

Table (5): Incidence of isolated coliforms from both types of Mozzarella cheese.

Mozzarella cheese / Isolated organism	Traditionally made No. = 15		Imported No. = 15	
	No.	%	No.	%
Coliform Isolates: E. coli	3	20	0	0
Klebsiella pneumoniae	6	40	1	6.67
K. oxytoca	2	13.33	1	6.67
K. ozaenae	2	13.33	0	0
K. rhinoscleromatis	1	6.67	0	0
Enterobacter aerogens	4	26.66	0	0
Enterobacter cloacae	1	6.67	1	6.67
Citrobacter diversus	4	26.66	0	0
Citrobacter freundii	3	20	0	0
Citrobacter amalonaticus	1	6.67	0	0

media (Enterococci selective differential media) by the technique described by (Efthymiou *et al*., 1974).

***Staphylococcus aureus* presumptive count**. It was made using Baird-Parker agar according to (Lancette and Tatini, 1993). The technique adopted by (Flowers *et al*., 1992) for isolation of *Salmonella species* was used by pre-enrichment in trypticase soya broth with yeast extract (TSBYE) followed by selective enrichment in Rappaport broth, and selective plating on XLD agar plates.

Results and Discussion

Although imported Mozzarella cheese contains starter culture, the mean total bacterial count (2.7×10^6CFU/g) is lower than that of traditionally made Mozzarella cheese using raw milk (8.5×10^7 CFU/g) (Table 1, 2, Fig.2). The highest frequency distribution was 40% for traditionally made type and 66.67% for imported type and lies within the range of 10^7- $<10^8$ and 10^6-$<10^7$ for both types respectively (Tables 3, 4). This may explain the high rate of contamination of the traditionally made Mozzarella cheese due to using raw milk in manufacturing process and the effect of using pasteurization process in reducing bacterial population in imported Mozzarella cheese before adding starter culture. These results were in agreement with that obtained by (Petracca *et al*., 1983; Warke *et al.,* 1999; Soliman, 2002).

Concerning psychrotrophic count, all examined Mozzarella cheese were positive for traditionally made cheese, with a minimum of 3×10^4 and maximum of $4.8\times10^7$with a mean count of $5.8\times10^5 \pm0.65\times10^5$, while for imported types only 8 samples (53.33%) were positive, the mean count was $3.7\times10^3 \pm 0.29\times10^3$.

The length of refrigerated storage (2°C) has a significant effect on psychrotrophic count which tend to increase (Sallam and Samejima, 2004; Fang *et al*., 2006). Psychrotrophic bacteria produce enzymes capable of hydrolyze proteins in Mozzarella cheese (Dervisoglu and Yazici, 2001).

Regarding coliforms in traditionally made Mozzarella cheese, the incidence was (100%) in all examined samples as compared with 2 samples (13.33%) for modern made Mozzarella cheese, with mean count (MPN/gram) for positive samples of $7.3\times10^4\pm0.95\times10^4$ CFU/g for traditionally made Mozzarella cheese, no statistical results were obtained for imported samples as the positive samples are too few (Tables 1, 2, Fig. 1, 2), the higher frequency distribution lies between 10^4 and $<10^5$ (53.34%) for traditionally made Mozzarella cheese (Table 3).

The counts for positive samples were much higher than limits suggested by Libyan Organization for Standardization for Mozzarella cheese (not exceeding 10 coliforms/g). Similar results were obtained by El-Prince and Ismail, (1998), while higher counts were obtained by Massa *et al.* (1992) and Soliman, (2002). Different coliform members were isolated in different percentages including *Klebsiella* species, *Enterobacter* species, *Citrobacter* species.

Escherichia coli was isolated from three samples (20%) of traditionally made Mozzarella cheese, while non was isolated from imported

type Mozzarella cheese (Table 5).

Presence of coliforms indicates unsanitary conditions of production, handling, distribution and neglected hygienic measures. In addition, many members of coliforms are responsible for gassy holes in Mozzarella cheese (Massa *et al.,* 1992), where *Klebsiella* species are responsible for spoilage and swelling of the plastic pouches for this type of cheese (Salvatore *et al*., 1992).

On the other hand, many *E. coli* strains were frequently associated with haemorrhagic colitis and haemolytic uraemic syndrome (HUS) (Griffin and Tauxe, 1991), and the presence of the organism is an indication of faecal contamination from human or animal sources (Armstrong *et al*., 1996) and contact with water and soil contaminated with ruminant manure (Coia, 1998 and Tozzi *et al*., 2001).

Staphylococcus aureus was present in 11 samples (73.33%) of traditionally made Mozzarella cheese, while present only in 4 samples (26.66%) of imported Mozzarella cheese with a mean count of 2.3×10^4 and 4.2×10^3 CFU/g in positive examined samples of both types respectively (Tables 1,2, Fig. 1,2). The highest frequency distribution of positive samples (20%) lies between 10^4 and $<10^5$ for the first type and lies between 10^3 and $<10^4$ for the later type (13.33%) (Tables 3,4). Nearly similar results were obtained by De Luca *et al.* (1997). Presence of *Staphylococcus aureus* in higher number may cause food poisoning due to production of enterotoxins (Tsung and Huang, 1993).

It is worth mentioning that the Libyan standards do not specify limits for such important organism count.

Enterococci was found in all examined Mozzarella cheese traditionally made while present in only 5 samples of imported samples (33.33%), the mean counts were 3.8×10^5 and 4.1×10^3 for both types respectively.

Salmonellae could not be detected in any of examined Mozzarella cheese samples of both types.

Most of Enterococci are relatively heat resistant and may survive milk pasteurization (Hartman *et al*., 1992). This may explain the higher incidence (33.33%) and count in imported types Mozzarella cheese as compared with other microbial groups for the same type of cheese. In addition most of Enterococci are also relatively resistant to freezing (APHA, 1992).

Presence of Enterococci is indicative for faecal contamination and consequently unsatisfactory production and handling (Moawad and El- Neary, 1996). Enterococci are also implicated in food poisoning (ICMSF, 1986) and can cause serious illness in human (Hoffman and Moellering, 1987).

Thus, it is important to focus the attention upon the quality of the raw milk before processing to ensure the quality of the end product made from it. Also, the unsatisfactory conditions of processing, handling and distribution of traditionally made Mozzarella cheese in addition to the lack of efficient veterinary supervision upon foods originated from animal origin may explain the obtained result in this work, therefore it is recommended to have specific standards for Mozzarella and other food products in Libyan Arab Jamahiriya for judging such product (s) and for supporting the food roles and laws, thus it is suggested to set the standards as quickly as possible.

In conclusion, the use of appropriate hygienic procedure, e.g. Hazard Analysis Critical Control Point system (HACCP) during processing should reduce the likelihood of higher microbial count and possibility of associated outbreaks.

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الجودة البكتريولوجية لجبن الموزريللا المعروض للبيع فى أسواق محافظة طرابلس

تم جمع ثلاثين عينة من جبن الموزريللا من أسواق مدينة طرابلس (١٥ عينة مصنعة محلياً وأخرى مستورده تحمل أسماء تجارية مختلفة) وذلك لغرض الفحص البكتريولوجي لتلك العينات والذى أشتمل على العدد الكلى للميكروبات، العدد الكلى للميكروبات المحبة للبرودة، العدد الأحتمالى للميكروبات القولونية ، العدد الأحتمالى للمكورات العنقودية الذهبية وكذلك العدد الكلى للمكورات المعوية. وقد كان متوسط الأعداد الميكروبية فى العينات المصنعة محلياً أعلى منها فى العينات المستوردة. هذا ولم يعزل ميكروب السالمونيلا من اى من العينات التى تم فحصها و تم عزل عدد من ميكروب الأيشيريكية القولونية الممرضة من بعض عينات الجبن الموزريللا المصنعة محلياً دون عزلها من الجبن المستورد. وقد كانت معظم العينات غير مطابقة للمواصفات القياسية المقترحة للعمل بها فى الجماهيرية الليبية.